EJFA Emirates Journal of Food and Agriculture

Emirates Journal of Food and Agriculture 37: 1–15
doi: 10.3897/ejfa.2025.129329

RESEARCH PAPER

Phenolic and amino acid profiles, and antioxidant properties of third generation snack foods adding *Citrus mitis* by-products and milk powder: impact of extrusion processing and microwave-heating "Impact of processing on obtaining healthy snacks"

Xóchitl Ariadna Ruiz-Armenta[1*], Carlos Iván Delgado-Nieblas[1*], José de Jesús Zazueta-Morales[1], Ernesto Aguilar-Palazuelos[1], Perla Rosa Fitch-Vargas[2], José Alberto Gallegos-Infante[3], José Ángel López-Valenzuela[1], María Fernanda Quintero-Soto[4]

1 *Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Sinaloa, Josefa Ortiz de Domínguez y Calzada de las Américas, Ciudad Universitaria, CP 80013 Culiacán, Sinaloa, Mexico*

2 *Facultad de Ciencias del Mar, Universidad Autónoma de Sinaloa, Paseo Claussen S/N, CP 82000, Mazatlán, Sinaloa, Mexico*

3 *TecNM/Instituto Tecnológico de Durango, CP 34080 Durango, Mexico*

4 *Universidad Politécnica del Mar y la Sierra, Carretera a Potrerillos del Norote Km 3, CP 82700 La Cruz, Sinaloa, Mexico*

Corresponding authors: Carlos Iván Delgado-Nieblas (cidelgadonieblas@uas.edu.mx); José de Jesús Zazueta-Morales (zazuetaj@uas.edu.mx)

Academic editor: Luca Rastrelli ♦ **Received** 17 June 2024 ♦ **Accepted** 9 December 2024 ♦ **Published** 6 February 2025

Abstract

Currently, there is a high consumption of snack foods worldwide, suggesting the improvement of their nutritional/nutraceutical properties by adding raw materials rich in bioactive compounds. This work aimed to study the impact of the processing stages on phenolic and amino acid profiles and the antioxidant properties of extruded snack foods by adding *Citrus mitis* by-products and milk powder. The color parameter C*, phytochemical, and antioxidant properties of four formulations were evaluated in the processing stages: unprocessed mixture (PS1), extruded pellets (PS2), and microwave-expanded products (PS3). The statistical analysis was performed using an unifactorial design with three levels (PS1, PS2, and PS3), evaluating four formulations (F1–F4) and a commercial product (COM). The mean values of the response variables were compared using the Fisher test ($p \leq 0.05$). Color parameter C* increased in PS3 concerning the PS2 and PS1 processing stages. Total flavonoid content (TFC), hydroxyl radical scavenging activity (HRSA), and inhibition of human LDL oxidation (ILDLox) decreased in PS2 regarding PS1. Likewise, TFC decreased in PS3 regarding PS2, whereas HRSA and ILDLox increased. PS3 exhibited higher TFC, HRSA, and ILDLox values in all formulations regarding commercial snack foods. Sixteen phenolic acids, nine flavonoids, and a hydroxy acid were identified in free and bound extracts by UPLC coupled to ESI-MS. The amino acid profile was affected by processing, and Lysine showed the lowest stability. The microwave-expanded snack foods had acceptable sensory properties evaluated by consumers, and adding raw materials rich in phytochemical compounds and high-quality proteins enhanced their nutraceutical/nutritional properties.

Keywords

Antioxidants, extrusion, healthy snack foods, microwave-heating, nutraceutical properties

* These authors contributed equally to this work.

Copyright *Ruiz-Armenta, et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC-BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Snack foods have become a crucial part of the daily diet in communities of all ages and social strata. Nonetheless, their high consumption can increase the likelihood of becoming overweight or obese and the risk of developing chronic diseases. Consequently, the demand for healthy and high-quality snack foods is growing (Maetens et al. 2017). Some of the most accepted and consumed snack foods are extruded snacks (directly or indirectly expanded). Indirectly expanded snack foods or third-generation snacks are high-density products with high storage stability. These snack foods can be expanded by frying in hot oil, puffing with hot air, or microwave heating (Panak-Balentić et al. 2018). The expansion using microwave heating may have an advantage regarding deep oil frying, giving lower-fat snack foods (Gümüşay and Şeker 2021). Commercial snack foods generally are based on corn, wheat, or rice starch. Snack foods are considered low nutritional quality due to their high energy density and poor nutrient content (junk food). However, to obtain healthy snacks has been recommended the addition of raw materials rich in bioactive compounds and dietary fiber, such as whole yellow corn (*Zea mays* L.), *Citrus mitis* B. fruit, as well as high-quality protein materials, such as milk powder (Ruiz-Armenta et al. 2019).

Diversifying methods for analyzing antioxidant activity in foods is imperative due to the varied mechanisms by which antioxidants function, including radical scavenging, hydrogen peroxide or hydroperoxide decomposition, transition metal ion sequestration, and active prooxidant quenching (Alam et al. 2013). DPPH and ABTS are the most popular colorimetric methods. However, another method that can be used is the deoxyribose method, which involves inhibiting the formation of hydroxyl radicals (OH^-). These radicals have the potential to cause significant biological harm, including cell death, mutation, and the development of cancer. Another important method for evaluating the antioxidant capacity *in vivo* is the inhibition of low-density lipoproteins (LDL). Free radicals commonly oxidize LDL, and these triggered reactions change its structure and functions (Rahman et al. 2015). Chronically elevated LDL cholesterol levels and oxidative modification of LDL are the principal causes of atherosclerotic plaque formation. Atherosclerosis is a progressive disease that consists of the thickening of the arterial wall due to the deposit of cholesterol, mainly LDL, causing a reduction in blood flow (Kiokias et al. 2018). The extrusion process is widely used to produce snack foods and can have different effects on the content of bioactive compounds and antioxidant activity. Brennan et al. (2011) reported that the extrusion process variables can positively or negatively influence the content of phenolic compounds and antioxidant activity. A decrease in the content of phenolic compounds can occur due to the decarboxylation of phenolic acids during the extrusion process because of factors such as temperature and moisture. Likewise, an increase in phenolic compounds due to the extrusion process has been reported (Zielinski et al. 2001; Brennan et al. 2011) since this process can release phenolic acids such as ferulic acid from cell walls, which can increase the antioxidant capacity. Currently, very little information has been found in the literature on determining the antioxidant activity in the different processing stages to obtain indirectly expanded snacks. Likewise, the amino acid content, inhibition of hydroxyl radicals (OH^-) formation, and inhibition of LDL oxidation in the different processing stages for the production of snacks have not been reported. Therefore, this investigation aimed to study the impact of the processing (extrusion and microwave heating) on the phenolic profile and antioxidants of healthy, indirectly expanded snack foods produced from whole yellow corn and *Citrus mitis* by-products. In addition, these products were compared with a commercial snack to determine the nutritional improvement achieved.

Materials and methods

Raw materials

The raw materials used for the production of indirectly expanded snack foods were corn starch (IMSA, S.A. de C.V., Mexico), whole yellow-corn (*Zea mays* L.) grains (SACSA S.A. de C.V., Navolato, Mexico), dehydrated *Citrus mitis* by-products (peels and segment walls), and skimmed milk powder (Wyeth, S. de R.L. de C.V.). The whole yellow-corn grains and dehydrated *Citrus mitis* by-products were ground individually (hammer-mill, Pulvex, model 200, Mexico City, Mexico) and sieved to obtain products with a particle size ≤420 µm. The raw materials were mixed using different concentrations to obtain four formulations (F1, F2, F3, and F4).

Snack foods production

Four formulations were made with corn starch (CS), whole yellow-corn flour (YF), *Citrus mitis* by-product flour (CM), and skimmed milk powder (SMP) in the first processing stage (unprocessed mixtures (PS1)). The first formulation (F1) contains CS and YF (60:40). The second formulation (F2) was performed with CS, YF, and CM (55.19:36.79:8.02). The third formulation (F3) is composed of CS, YF, CM, and SMP (56.40:37.60:6.0), and the fourth formulation has CS, YF, and SMP (51.59:34.39:8.02). Each PS1 was subjected to the extrusion process using the following barrel temperatures (BT): food zone = 75 °C, central zone = 125 °C, molding zone = 75 °C. Likewise, the feed moisture was 23 g of water/100 g of sample, and the screw speed was 75 rpm. A single screw (1:1 ratio) laboratory extruder (Brabender 20DN, model 8-235-00, O-HG Brabender, Duisburg, Germany) with a rectangular die (20 mm wide, 1.0 mm high, 100 mm long) was employed to obtain the extruded pellets (PS2) of 3 cm long that subsequently were dehydrated at 25 °C for 72 h. Finally, the extruded pellets were expanded by microwave

heating (MS1149CS model, LG, Mexico, at 1450 W and 2450 MHz) for 20 s to obtain the expanded snacks (PS3) and analyzed (Ruiz-Armenta et al. 2019). Fig. 1 shows the different processing stages used to produce the snack foods. Additionally, the four snack formulations were compared with a commercial product (COM) based on potato starch (Comercializadora Viridiana, S.A. de C.V., Jiutepec, Mexico) in its third processing stage (EXP). The criteria for selecting the commercial snack were the type of snack (pellet) and the shape (rectangular) of the product because no pellets with the addition of raw materials rich in bioactive compounds were found.

Color parameters (C*, h*)

The chroma (C*) and hue angle (h*) color parameters were measured with a tristimulus colorimeter (Minolta, CR-210, Tokyo, Japan). The samples were ground to a particle size ≤250 µm and subsequently placed in 5 cm diameter Petri dishes, taking three equidistant measurements. Five replicates were made per treatment.

Phenolic extraction

This extraction was performed according to Adom and Liu (2002). The free fraction (FF) was extracted using ethanol solution (80 mL/100 mL), followed by centrifugation (3000 *xg*/10 °C/ 10 min) and concentration (40 °C). The concentrate was dried using an oven and reconstituted using 2 mL of methanol solution (50 mL/100 mL). Likewise, the residue was used to obtain the bound fraction (BF) using digestion with NaOH and neutralization with HCl. After that, hexane was added, and centrifugation (3000 *xg*/10 °C/ 10 min) was carried out, eliminating the supernatant. Ethyl acetate was added, and the extracted compounds were reconstituted with 2 mL of methanol solution (50 mL/100 mL) to determine the bound phenolic compounds. The FF and BF extracts were used to measure the flavonoid and phenolic compounds content and the antioxidant activity of samples. Three replicates were made per treatment.

Flavonoids content (FC)

The total flavonoid determination was performed by mixing the methanolic extract (FF and BF), distilled water, and $NaNO_2$ (5 g/100 mL). Later, $AlCl_3$ (10 g/100 mL) and NaOH 1M were added. The absorbance was read at 510 nm (UV-GENESYS model 10, Series AQ7-2H7G229001, Thermo Electron Scientific Instruments LLC, Madison, Wisconsin, USA), and the results were expressed as mg quercetin equivalents (QE)/g sample dry basis (db). The total flavonoid content was calculated through the sum of the two fractions (FF and BF) (Dewanto et al. 2002). Three repetitions were made per treatment.

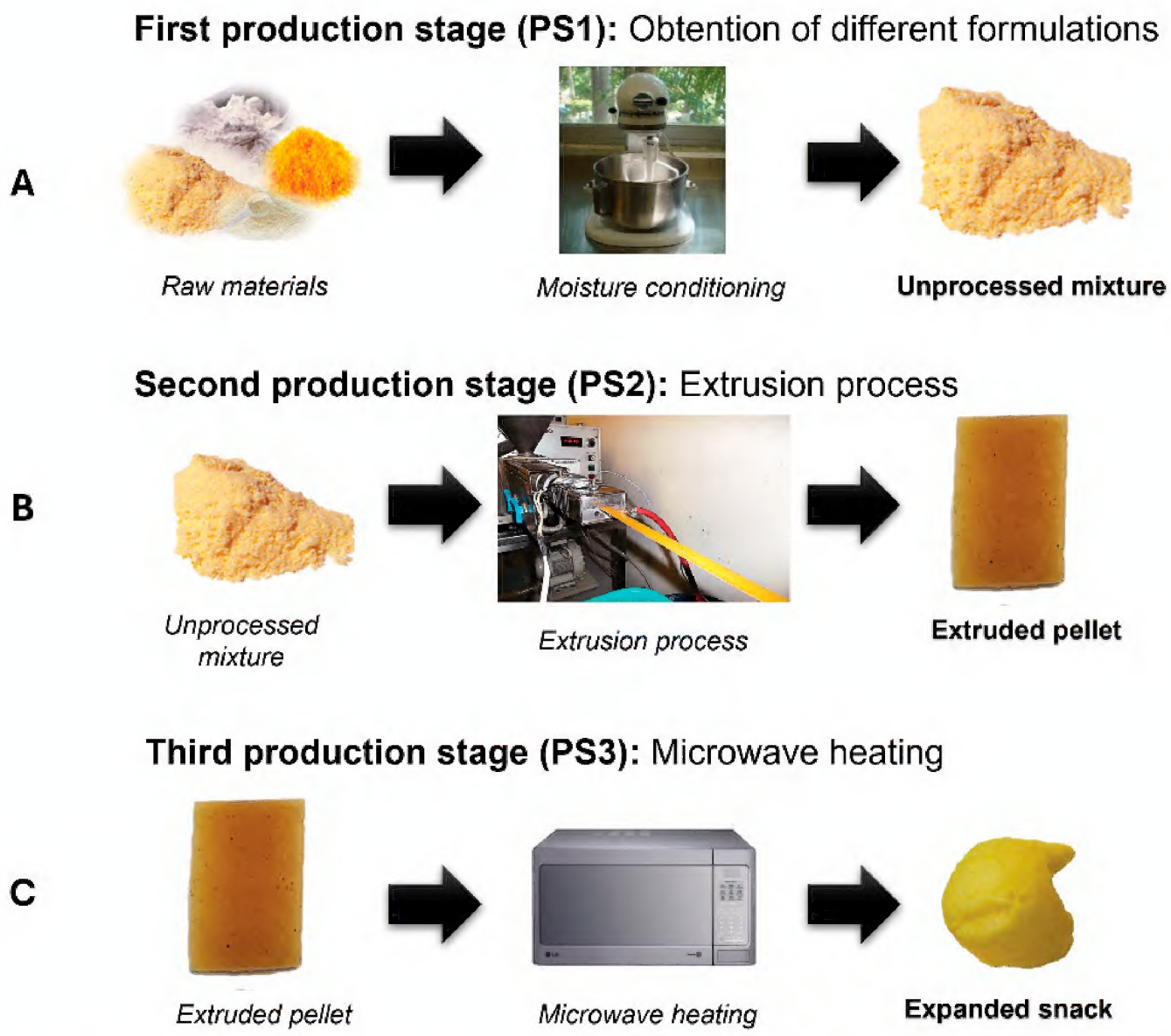

Figure 1. Processing stages to obtain indirectly expanded snack foods and their respective products. (**A**) first production stage = unprocessed mixture (PS1), (**B**) second production stage = extruded pellet (PS2), (**C**) third production stage = expanded snack (PS3).

Identification of phenolic compounds by UPLC–DAD–ESI-MS

An aliquot of 10 µL of each extract (free and bound fractions) was injected into an UPLC–DAD system (ACCELA, Thermo Fisher Scientific Inc., Waltham, MA) connected to an LTQ XL mass spectrometer (Thermo Scientific, Waltham, MA). The separation was performed in a C_{18} column (3 µm, 50 × 2.1 mm) (Fortis Technologies Ltd., Neston, Cheshire, United Kingdom). The mobile phase consisted of acidified water by formic acid (solvent A, 1 mL/100 mL) and acetonitrile (solvent B), following a linear gradient from 0.5 to 60% (60 mL/100 mL) of B for 40 min to 0.2 mL/min. The detection was recorded at 280, 320, and 350 nm. The mass spectrometer system was equipped with an electrospray interface (ESI) operating in negative and positive ionization modes, with a capillary voltage and temperature of 35 V and 300 °C, respectively. A software Xcalibur 2.2 (Thermo Fisher Scientific Inc., Waltham, MA) in full-scan mode was used within the range of m/z 110–2000. The identification of phenolic compounds was based on retention time and mass spectra data. The peaks were identified by comparing the obtained information with available data reported in the literature (Quintero-Soto et al. 2018).

Hydroxyl radical scavenging activity (HRSA)

The hydroxyl radical (OH^-) scavenging through the deoxyribose degradation assay was evaluated (Gamboa-Gómez et al. 2016). A mixture of deoxyribose (28 mM), $FeCl_3$ (0.1 mM), ethylenediaminetetraacetic acid (0.1 mM), H_2O_2 (10 mM), ascorbic acid (1 mM) and the sample (1 mg/mL) in phosphate buffer (50 mM, pH 7.4) solution, were incubated for 1 hour at 37 °C. Subsequently, trichloroacetic acid (2.8 g/ 100 mL) and thiobarbituric acid (1 g/100 mL) were added. The reactions were mixed and heated in a water bath at 95 °C for 20 min. Three replicates were made per treatment. The organic phase was read at 532 nm. HRSA was calculated as the inhibition (%) of OH radicals using the following equation:

$$\%HRSA = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (1)$$

Inhibition of human low-density lipoprotein oxidation (ILDLox)

The low-density lipoproteins (LDL) were precipitated from human blood plasma using a kit (HDL cholesterol P-precipitating reagent, SPINREACT brand, Girona, Spain). The oxidation catalyzed by copper (25 mM/L) was evaluated by incubating the materials at 37 °C/3 h. The number of peroxides was evaluated using the thiobarbituric acid method (Gamboa-Gómez et al. 2016). The absorbance was read at 532 nm, and the ILDLox was expressed as a percentage. Three repetitions were made per treatment. The inhibition was calculated using the following equation:

$$\%ILDLox = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (2)$$

Essential amino acid profile

This analysis was performed on two production stages (unprocessed mixture (PS1) and expanded product (PS3)) obtained in the third formulation (F3). MME-AA-01 and MME-AA-02 ion-exchange chromatography and MME-AA-03 visible ultraviolet spectrophotometry were used (Morales-De León et al. 2005).

Sensory analysis

The sensory acceptability of snacks (PS3) from the formulations F3 and F4 was evaluated at a room temperature of 25 ± 2 °C under normal lighting conditions using a 9-point hedonic scale, where (1 = I dislike extremely, 2 = I dislike a lot, 3 = I dislike moderately, 4 = I dislike slightly, 5 = I do not like or dislike, 6 = I like slightly, 7 = I like moderately, 8 = I like a lot, and 9 = I like extremely) (Lim 2011). A total of 180 untrained panelists were selected to evaluate the snack foods. The main criteria for selection were that participants were over 17 years old and enjoyed consuming snack foods. All panelists were instructed to observe, smell, and taste each snack food. They were asked to fill out a questionnaire evaluating the impact of the nutritional information of the snack foods on their overall acceptance and acceptance by evaluating the sensory attributes of color, flavor, and texture. Each evaluation questionnaire included a section for panelists to provide observations on each formulation (F3, F4, COM).

Statistical analysis

The statistical analysis was carried out in two parts. An unifactorial experimental design where the factor was the processing stage to obtain the indirectly expanded snack foods, and its three levels were the unprocessed mixture (PS1), extruded pellet (PS2), and expanded product (PS3) was used in the first part of the study. Likewise, an unifactorial design with five levels was used in the second part of the study. The factor was the type of expanded snack food, and the levels were the formulations F1, F2, F3, F4, and a commercial product (COM). The means comparisons were performed with the Fisher test (LSD, $p \leq 0.05$) using the Design Expert 7.0 statistical software. Pearson correlations were performed using Statistica 7.0 software (Statsoft, Tulsa, OK, USA).

Results and discussion

Raw materials

The raw materials that presented the highest content of flavonoids and antioxidant activity were *Citrus mitis* by-products flour and whole yellow-corn flour. The *Citrus mitis* by-product flour presented the highest values for the color parameter C* (37.51 ± 0.82) and total flavonoids (2.00 ± 0.18 mg QE/g sample db), where the free fraction (FF) exhibited the main flavonoid content (1.98 ± 0.18 mg QE/g sample db), as well a high OH^- radical inhibition (HRSA, FF = 41.82 ± 2.90%, and in bound fraction (BF)= 31.82 ± 1.09%) and inhibition of human low-density lipoprotein oxidation (ILDLox, FF = 59.31 ± 3.17%, and BF = 32.17 ± 2.15%). On the other hand, a value of color parameter C* of 24.70 ± 1.91 was found in whole yellow corn flour. Also, in this raw material, the bound fraction (BF) presented the highest total flavonoid content (0.94 ± 0.09 mg QE/g sample db) and antioxidant activity (50.27 ± 2.42% HRSA and 64.85 ± 4.02% ILDLox). Adom and Liu (2002) found similar behavior, observing a high correlation between total antioxidant activity, total phenolic compounds, and total flavonoids in cereals (oat, wheat, rice, and corn). In that work, the authors reported that the FF contributed 13%, and the BF contributed 87% of the total antioxidant activity. Also, the above authors mention that in whole grains, bound phenolics are associated with cell wall compounds that may resist gastrointestinal digestion and reach the colon. Colonic digestion, by intestinal microflora, of such materials may liberate the bulk of bound phytochemicals.

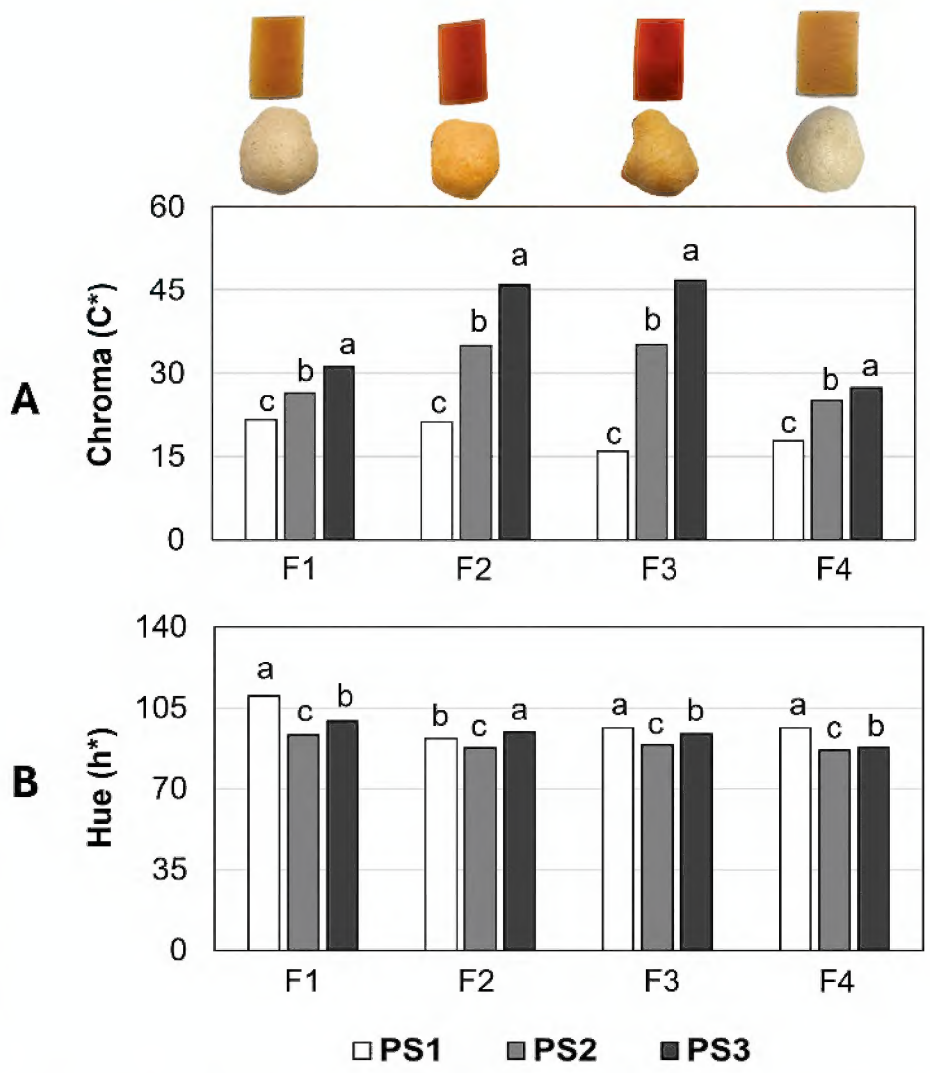

Figure 2. Color parameters C* (**A**) and hue (**B**) as an effect of the different processing stages (PS1 = unprocessed mixture, PS2 = extruded pellet, and PS3 = expanded snack) of indirectly expanded snack foods. [a-c] Different letters between processing stages show a significant difference (LSD, $p < 0.05$).

Color parameters

Formulations F2 (C*= 45.78 ± 0.39, h*= 94.57 ± 0.24) and F3 displayed a more pronounced yellow-orange hue on PS3 (C*= 46.75 ± 0.85, h*= 93.73 ± 0.15), contrasting with the lighter yellow appearance of formulations F1 (C*= 31.01 ± 0.19, h*= 99.35 ± 0.25) and F4 (C*= 27.52 ± 0.77, h*= 88.01 ± 0.38). While the commercial product presented values of C*= 20.05 ± 0.21 and h*=86.34 ± 0.25) (Fig. 2). This disparity in color intensity likely stems from inherent variations in the raw materials utilized. Starch and powdered milk have light colors (white-beige), yellow-corn flour has a light-yellow color, and *Citrus mitis* by-product flour has a yellow-orange color. C* quantifies color saturation, where higher values indicate increased color intensity perceptible to the human eye. The h* parameter denotes specific color differences relative to a gray reference with equal luminosity. An angle of 0° or 360° signifies a red hue, while hues of yellow, green, and blue correspond to angles of 90°, 180°, and 270°, respectively.

Flavonoids content (FC)

Flavonoids are polyphenolic compounds found in fruits, vegetables, and cereals. Consuming flavonoid-rich foods and nutraceuticals has been linked to various health benefits. People who consume foods rich in these compounds have been observed to have a lower incidence of diseases such as diabetes, cancer, inflammation, and neurodegeneration. These benefits are attributed to the antioxidant, antimicrobial, and antiviral properties of flavonoids, and their ability to neutralize free radicals and inhibit crucial enzymes (Safe et al. 2021). The World Health Organization (WHO) recommends consuming antioxidant-rich foods as a preventative measure against chronic diseases. Nonetheless, no official guidelines specify the types, number, or daily amounts of antioxidants required (Poljsak et al. 2021). F2 and F3 exhibited the highest values for the FC (Fig. 3A). These results could be because flavonoids and phenolic acids are the most abundant polyphenols in citrus by-products, such as *Citrus mitis*. Flavonoids are mainly present in the peel as aglycones or glycosides (C- or O-glycosides), the latter being the dominant form. In addition, the flavonoid content (free flavonoid fraction and total flavonoids (TF)) decreased in PS2 regarding PS1, mainly in F2 and F3. This effect correlates significantly with the content of free ($r = 0.85$, $p < 0.01$) and total ($r = 0.75$, $p < 0.01$) phenolic compounds reported in a previous study (Ruiz-Armenta et al. 2019). The decarboxylation, decomposition, or depolymerization of these phytochemical compounds can be carried out by the high temperatures and shear during the extrusion process (Xu et al. 2016). The behavior and total flavonoid values presented by the F2 and F3 (with

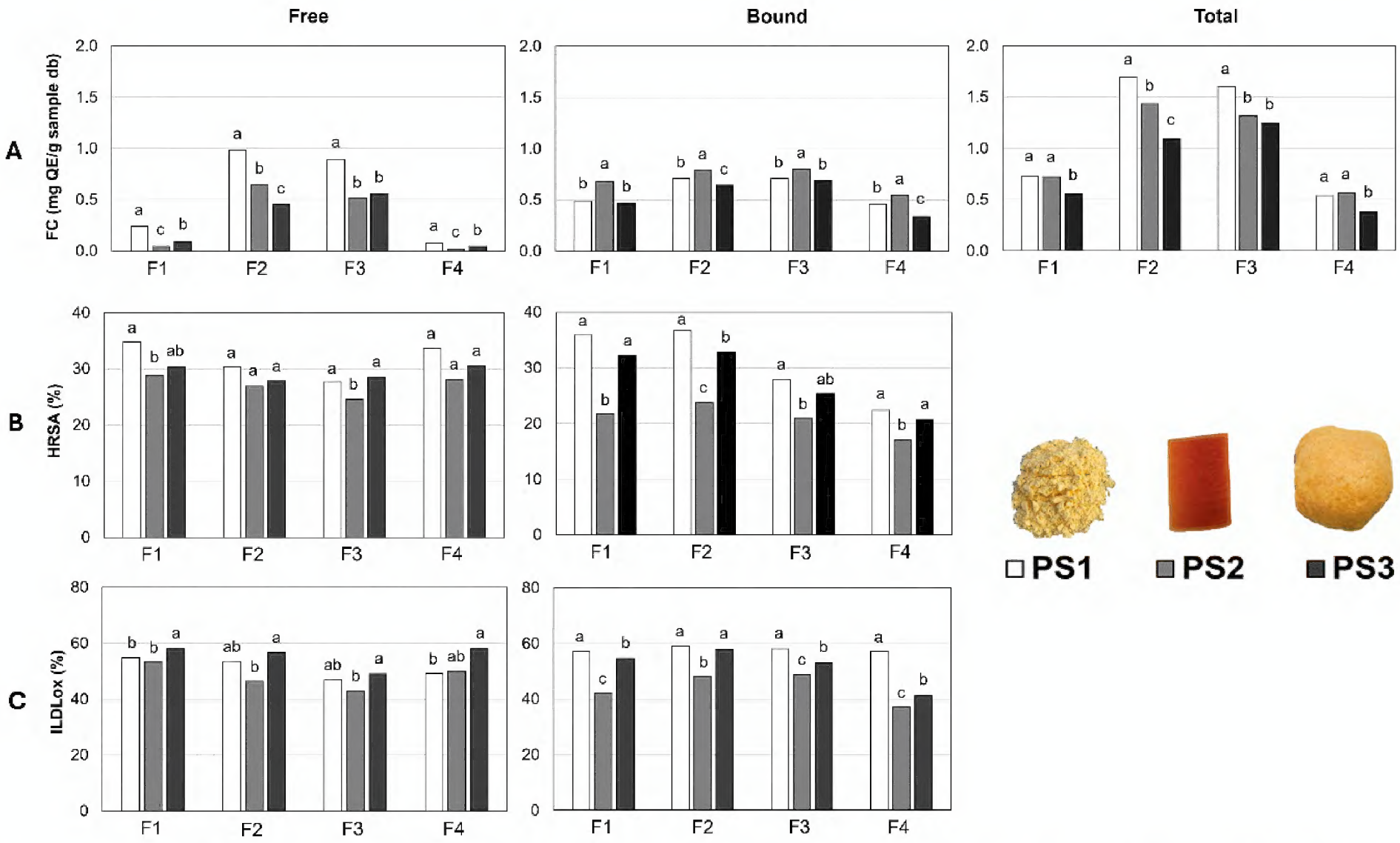

Figure 3. Flavonoids content (free, bound, and total fractions) (**A**), hydroxyl radical scavenging activity (free and bound fractions) (**B**), and inhibition of human LDL oxidation (free and bound fractions) (**C**) as an effect of the different processing stages for the production of indirectly expanded snack foods (PS1 = unprocessed mixture, PS2 = extruded pellet, and PS3 = expanded snack). [a-c] Different letters between processing stages show a significant difference (LSD, $p < 0.05$).

Citrus mitis by-products) are similar to the obtained in the work of Wani et al. (2021), who reported that the extrusion cooking reduced the values of total flavonoid content from 1003 ± 13 to 802 ± 9 µg catechin equivalents/g in snack foods enriched with a multi-component mixture of cereals and legumes, attributing this reduction to heat damage in the flavonoid compounds. Patil et al. (2016) observed a significant decrease ($p < 0.05$) in the total flavonoid content when finger millet and sorghum flours were subjected to the extrusion process. The non-extruded materials presented values of 214.67 mg quercetin equivalents (QE)/100 g (db.) and 21.18 mg QE/100 g (db.) for finger millet and sorghum flour, respectively. However, the extrusion process decreased the total flavonoid values to 110.39 and 8.75 mg QE/100 g (db.) in finger millet and sorghum, respectively. The authors attribute this decrease to the sensitivity of flavonoids to heat, highlighting that their thermal stability depends on several factors, such as the nature of the matrix, the type of processing, and the temperatures applied. Also, the bound flavonoids fraction (BFF) increased in PS2 regarding PS1. Also, disrupting the cell walls by the thermomechanical extrusion process can improve the extraction of BFF. Moreover, TF decreased in PS3 regarding PS2. According to Routray and Orsat (2012), using high microwave power values could raise the temperature of the product, causing damage to food components such as flavonoid compounds. Nonetheless, in stage PS3, all formulations (F1, F2, F3, and F4) presented higher significantly values ($p < 0.05$, LSD = 0.05) of TF (0.55 ± 0.05, 1.09 ± 0.03, 1.25 ± 0.03, 0.38 ± 0.02 mg QE/g sample db, respectively) concerning the commercial product (COM, 0.32 ± 0.02 mg QE/g sample db).

Hydroxyl Radical Scavenging Activity (HRSA)

The OH^- radical neutralization in living systems has important health benefits and is crucial to survival. The neutralization is desirable due to its high reactivity and can occur in materials with high antioxidant activity, such as flavonoids. The OH^- is the most reactive free radical formed in the body, with an estimated production of 50 per second per cell. It is considered the most damaging free radical species, as it attacks any molecule in its immediate proximity (Martemucci et al. 2022).

The OH^- radical can react with an extensive gamma of molecules in living cells, such as sugars, lipids, amino acids, and nucleotides. The OH^- radicals formation may occur in diverse ways. The Fenton reaction is the primary mechanism *in vivo*. A transition metal participates as a prooxidant in the catalyzed decomposition of superoxide and hydrogen peroxide (Hsu et al. 2006).

In all formulations (F1, F2, F3, and F4), the stage PS1 (free fraction (FF) and bound fraction (BF)) presented high values of HRSA (Fig. 3B). Likewise, the HRSA (FF and BF) values decreased in PS2 concerning PS1, except in

formulations F2 and F4 of FF, where no significant difference was presented. Also, a tendency to increase the HRSA values (FF and BF) in stage PS3 concerning PS2 was presented. No significant difference between PS3 and PS1 was found, except for F2 (BF). The reduction of HRSA in PS2 concerning PS1 could be because the extrusion process degraded the phenolic compounds through decarboxylation reactions since they are heat-labile (Wani and Kumar 2016). The HRSA values presented in the present study in the extruded product (PS2) of different formulations for BF and FF are in the range of the HRSA values (15.14%–83.56%) reported by Qiao et al. (2021) in extruded products, who observed that the extrusion process increased ($p < 0.05$) the HRSA values compared to non-extruded (10.74%–61.34%) sweet potato residues at all concentrations analyzed ($p < 0.05$). Likewise, the HRSA values obtained in the present study in PS2 were higher than the values obtained by Wang et al. (2020), who observed a significant increase ($p < 0.05$) in the HRSA values (from 5.3% to 15.9%) when they subjected wheat bran to the extrusion process. According to these authors, the increase in HRSA could be due to the content of phenolic acids and soluble dietary fiber provided by the bran. Rocha-Guzmán et al. (2012) found the highest HRSA values in the treatments that presented higher content of yellow corn and high extrusion temperature in snack foods based on yellow-corn flour and Cehualca pumpkin flour. The authors attributed this behavior to the high content of yellow-corn flour, its phytochemical composition (highlighting the presence of ferulic acid), and its higher radical scavenging capacity. Also, the increase in HRSA of FF in PS3 concerning PS2 can be related to the content of phenolic compounds (phenolic acids, flavonoids) (Ruiz-Armenta et al. 2019), where the microwave treatment could have liberated phenolic compounds, causing an increase in antioxidant activity. The liberation of phenolic compounds could be due to the applied microwave power and the residence time inside the microwave oven. Also, thermal treatments such as microwave heating could increase the content of bound phenolics since phenolic compounds can form complexes with proteins and fiber, increasing the HRSA of BF. Hence, the extruded snacks (F1, F2, F3, and F4) at the different processing stages (PS1, PS2, PS3) recorded high values of HRSA (>20%). All expanded snacks (PS3) presented higher significant values of HRSA ($p < 0.05$, LSD = 3.9 in free fraction and LSD = 4.5 in bound fraction) than the commercial product (COM, <5%).

Determination of the biological effect inhibition of human low-density lipoprotein oxidation (ILDLox)

Cardiovascular diseases (atherosclerosis and its acute coronary syndromes) rank among the leading causes of death worldwide. However, these diseases can be prevented by adopting a healthy lifestyle (WHO 2020) and consuming a diet with high levels of antioxidants. According to Gąsecka et al. (2021), LDLox are derived particles of circulating LDL with peroxides or their degradation products linked with the LDL particle. LDLox has pro-thrombotic properties and plays a key role in atherosclerosis due to acts on diverse cells such as endothelial cells, macrophages, platelets, fibroblasts, and smooth muscle cells. Atherosclerosis and its acute coronary syndromes are among the leading causes of death worldwide. Therefore, searching for alternatives for inhibiting human low-density lipoproteins oxidation is essential. The intake of snack foods with antioxidant compounds that inhibit LDLox may suggest that they are health-promoting foods since the snacks typically contain high amounts of salt and fat. Values >35% of ILDLox were obtained in all treatments (Fig. 3C). Moreover, >40% of ILDLox were found in the PS3 stage in both extracts (FF and BF). The ILDLox decreased in PS2 regarding PS1 (FF and BF). This behavior coincides with the results obtained in HRSA. It has been reported that phenolic acids subjected to high temperature and moisture contents can be degraded through decarboxylation (Buitimea-Cantúa et al. 2018). The extrusion process can induce structural changes in phenolic compounds for the different parameters of extrusion (temperature, shear inside the barrel, pressure, etc.) (Ruiz-Armenta et al. 2019). The lowest ILDLox values (38–42%) were found in PS2 (BF). Also, significant differences were found between PS1 and PS3, except for F2 in the BF. The increase in ILDLox in the PS3 products could be because the electromagnetic radiation emitted by the microwave oven when the snacks were expanded caused a release of the phenolic compounds (phenolic acids or flavonoids) from the matrix where they were located and, therefore, an increased antioxidant activity (ILDLox), by elevating the radical trapping action. The antioxidant activity depends on the concentration and bioactive compound structure. A structure/function relationship could exist among the several fractions/constituents linked to the flavonoid three-ring system and their effect on LDL oxidation (LDLox). Hydroxycinnamic acid derivatives, such as ferulic acid, highly present in cereals such as corn, are efficient against Cu^{2+}-induced LDL peroxidation since molecules containing ortho-dihydroxyl or 4-hydro-3-methoxyl groups have significantly higher antioxidant activity (Cheng et al. 2007). Also, in the FF, no significant differences ($p > 0.05$, LSD = 1.7) were found in all the PS3 samples, whereas the F2 in the BF obtained the highest ILDLox (57.9 ± 1.41%). All snacks in the stage F3 showed higher ILDLox ($p > 0.05$, LSD = 1.7) than the commercial product (COM, <1.7%).

The ILDLox values obtained in the present study in the extruded product (PS2) of different formulations for BF are similar to the values reported by Castro-Montoya et al. (2024) in extruded gluten-free pasta using broken rice and nopal flour as raw materials, while the values obtained in PS2 for FF were slightly lower. The difference in values may be due to the raw materials and the type of processing used for their production. In the work mentioned above, the higher values were obtained at high extrusion temperatures and nopal flour content. The results were attributed to the phenolic compounds content, such as

acid phenolics and flavonoids mainly provided by nopal flour, which were released by the extrusion temperatures, increasing the antioxidant activity (ILDLox). Rocha-Guzmán et al. (2012) reported a significant inhibition of LDLox in indirectly expanded snacks, attributed to the high pumpkin content and elevated extrusion temperatures. The authors linked these findings to the presence of gallic acid and catechin. Moreno-Jiménez et al. (2015) identified several bean varieties with solid potential to inhibit LDLox, particularly noting that the Black 8025 variety could achieve 50% inhibition at extract concentrations below 1000 ppm. Based on the results obtained for flavonoid content and antioxidant capacity (HRSA and ILDLox), along with findings from a previous study (Ruiz-Armenta et al. 2019) where the formulations F3 and F4 presented high expansion values, dietary fiber, and phenolic compounds, were identified the specific phenolic compounds present in snack foods for F3 and F4, and evaluated the sensory acceptability of these products.

Phenolic compounds profile

Two samples were selected to evaluate the phenolic profile. Formulations 3 and 4 were chosen due to their high content of phenolic compounds and their physical and physicochemical properties, which were like commercial products, as observed in a previous study (Ruiz-Armenta et al. 2019). The results revealed the presence of a wide variety of bioactive compounds, highlighting the richness of the phenolic profile of the extruded snacks enriched with *Citrus mitis* and yellow corn by-products. Sixteen phenolic acids, nine flavonoids, and a hydroxy acid were identified in free and bound extracts by UPLC coupled to ESI-MS. Table 1 shows the retention time, fragmentation patterns, fragment descriptions, identified phenolic compounds, and their classification. Among the identified phenolic acids, thirteen were categorized as hydroxycinnamic acids (caffeoyl hexose I, caffeoyl-hexose II, caffeoylquinic acid hexoside, chlorogenic acid, caffeoyl-hexose III, *p*-coumaric acid, ferulic acid hexoside, ferulic acid, isoferulic acid, diferulic acid, hydrotriferulic acid (cyclic), caffeoyl-hexose-deoxyhexose, hydrodiferulic acid). Also, the remaining three phenolic compounds belonged to the hydroxybenzoic acids (caffeic acid, syringic acid hexoside, and syringic acid). Likewise, the flavonoids identified were two flavones (5-hydroxy-penta-methoxy-flavone I and tangeretin), one flavonol (Rutin), and six flavanones (Neoeriocitrin I, Neoeriocitrin II, Narirutin, Naringenin hexoside I, Hesperidin, Naringenin hexoside II). Some of the characteristic fragments of phenolic acids included m/z 193 $[M\text{-}H]^-$ for ferulic acid, m/z 179 $[M\text{-}H]^-$ for caffeic acid, m/z 271 $[M\text{-}H]^-$ for naringenin, m/z 303 $[M\text{-}H]^-$ for hesperidin, and m/z 609 $[M\text{-}H]^-$ for rutin, confirming their presence. The compounds isoferulic acid, rutin, 5-hydroxy-penta-methoxy-flavone I, and tangeretin (peaks 13, 18, 22, and 25, respectively) were present in F3 but absent or in lower proportions in F4. In F3, the predominant phenolic compound was naringenin hexoside I, while in F4, ferulic acid predominated (Fig. 4). Rao et al. (2017) reported that naringenin has antioxidant, anti-inflammatory, and DNA repair properties. They found a reduction in DNA damage by the hydroxyl radical by 24% at a concentration of 80 mM in 24 hours. At the same time, ferulic acid exhibits antioxidative, anti-inflammatory, antifibrotic, and vascular endothelial protective effects (Li et al. 2021).

Significant changes in phenolic compounds were observed after the extrusion process and microwave heating. In F3, when the unprocessed mixture (PS1) underwent extrusion process (PS2), new peaks (21, 23, and 26) appeared in the chromatograms, which were absent in PS1. Also, the microwave heating to obtain the expanded product (PS3) further increased the area of these peaks (Fig. 5). The compounds corresponding to these peaks were diferulic acid, hydrotriferulic acid (cyclic), and hydrodiferulic acid. Zeng et al. (2016) reported significant changes in the content of various phenolic acids following extrusion processing. According to the authors, phenolic acids bound to cell walls, such as ferulic acid, can be converted into free phenolic acids during thermal processing, facilitating their extraction. *p*-Coumaric acid, ferulic acid, naringenin hexoside II, and diferulic acid were consistently detected across all processing stages in both formulations, F3 and F4, albeit in varying proportions. Whereas ferulic acid hexoside, 5-hydroxy-penta-methoxy-flavone I, and tangeretin were exclusively found in the *Citrus mitis*-containing product (F3), manifesting in differing proportions across various processing stages. Different phenolic compounds have been identified in citrus fruits. Moulehi et al. (2012) reported the presence of diverse flavonoids in unripe and ripe mandarin, among which are naringin, hesperidin, catechin, quercetin, kaempferol, as well as gallic, vanillic, rosmarinic, *p*-coumaric acid, among others. Lou et al. (2014) found naringin, hesperidin, nobiletin, tangeretin, diosmin, and 3',5'-di-C-β-glucopyranosylphloretin in *Citrus mitis*. Also, in cereals such as maize, the mainly reported phenolic compounds are ferulic and *p*-coumaric acids (Žilić et al. 2012). In addition, Gaxiola-Cuevas et al. (2017) reported that the main phenolic acids found in pigmented corn were *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, sinapic, and ferulic. Therefore, it is convenient to add *Citrus mitis* by-products in the production of snack foods due to the wide variety of phenolic compounds with antioxidant potential that provide these by-products.

Sensory analysis

The sensory properties of foods are crucial since they indicate their acceptability by consumers. This study evaluated the impact of information on the overall acceptance of snack foods. When the snacks were evaluated without providing prior nutritional information to participants, F3 demonstrated an overall acceptance of 67%, F4 exhibited

Table 1. Phenolic compounds identified in methanol extracts of indirectly expanded snack foods (F3 and F4) using UPLC–DAD–ESIMS.

No.	RT (min)	Fragment ion	Fragment description	Identified compound	Compound type	Reference
1	11.13	179 [180-H]$^-$	Deprotonated molecular ion	Caffeic acid	Hydroxybenzoic acid	(Quintero-Soto et al. 2022)
		135 [180-H-44]$^-$	Loss of a CO_2 molecule			
2	12.03	341 [342-H]$^-$	Deprotonated molecular ion	Caffeoyl hexose I	Hydroxycinnamic acid	(Pineda-Hidalgo et al. 2022)
		179 [342-H-162]$^-$	Loss of a hexose			
		135 [342-H-162-44]$^-$	Loss of a CO_2 molecule			
3	13.3	137 [138-H]$^-$	Deprotonated molecular ion	Salicylic acid	beta-hydroxy acids	(Pineda-Hidalgo et al. 2022)
		93 [138-H-44]$^-$	Loss of a CO_2 molecule			
4	13.8	341 [342-H]$^-$	Deprotonated molecular ion	Caffeoyl-hexose II	Hydroxycinnamic acid	(Pineda-Hidalgo et al. 2022)
		179 [342-H-162]$^-$	Loss of a hexose			
		135 [342-H-162-44]$^-$	Loss of a CO_2 molecule			
5	14.71	515 [516-H]$^-$	Deprotonated molecular ion	Caffeoylquinic acid hexoside	Hydroxycinnamic acid	(Barraza-Elenes et al. 2019)
		353 [516-H-162]$^-$	Loss of a hexose			
		191 [516-H-162-162]$^-$	Loss of remains of a caffeoyl molecule			
6	15.19	353 [354-H]$^-$	Deprotonated molecular ion	Chlorogenic acid	Hydroxycinnamic acid	(Simirgiotis et al. 2015)
		191 [354-H-162]$^-$	Loss of remains of a caffeoyl molecule			
7	17.06	359 [360-H]$^-$	Deprotonated molecular ion	Syringic acid hexoside	Hydroxybenzoic acid	(Félix-Medina et al. 2021)
		197 [360-H-162]$^-$	Loss of a hexose			
8	17.77	197 [198-H]$^-$	Deprotonated molecular ion	Syringic acid	Hydroxybenzoic acid	(Wang et al. 2022)
		153 [198-H-44]$^-$	Loss of a CO_2 molecule			
9	18.28	341 [342-H]$^-$	Deprotonated molecular ion	Caffeoyl-hexose III	Hydroxycinnamic acid	(Pineda-Hidalgo et al. 2022)
		179 [342-H-162]$^-$	Loss of a hexose			
		135 [342-H-162-44]$^-$	Loss of a CO_2 molecule			
10	23.06	163 [164-H]$^-$	Deprotonated molecular ion	*p*-Coumaric acid	Hydroxycinnamic acid	(Barraza-Elenes et al. 2019)
		119 [164-H-44]$^-$	Loss of a CO_2 molecule			
11	25.18	355 [356-H]$^-$	Deprotonated molecular ion	Ferulic acid hexoside	Hydroxycinnamic acid	(Ramírez et al. 2020)
		193 [356-H-162]$^-$	Loss of a hexose			
		149 [356-H-162-44]$^-$	Loss of a CO_2 molecule			
12	25.82	193 [356-H]$^-$	Deprotonated molecular ion	Ferulic acid	Hydroxycinnamic acid	(Barraza-Elenes et al. 2019)
		149 [356-H-44]$^-$	Loss of a CO_2 molecule			
13	26.24	193 [356-H]$^-$	Deprotonated molecular ion	Isoferulic acid	Hydroxycinnamic acid	(Barraza-Elenes et al. 2019)
		149 [356-H-44]$^-$	Loss of a CO_2 molecule			
14	27.11	595 [596-H]$^-$	Deprotonated molecular ion	Neoeriocitrin I	Flavanone	(Xu et al. 2022)
		433 [596-H-162]$^-$	Loss of a hexose			
		283 [596-H-162-150]$^-$	Loss of remains of a hexose molecule			
15	27.74	595 [596-H]$^-$	Deprotonated molecular ion	Neoeriocitrin II	Flavanone	(Xu et al. 2022)
		433 [596-H-162]$^-$	Loss of a hexose			
		283 [596-H-162-150]$^-$	Loss of remains of a hexose molecule			
16	28.55	579 [580-H]$^-$	Deprotonated molecular ion	Narirutin	Flavanone	(Xu et al. 2022)
		271 [580-H-308]$^-$	Loss of a rutinoside group			
		151 [580-H-308-157]$^-$	Derived from the retro Diels-Alder mechanism			
17	28.99	433 [434-H]$^-$	Deprotonated molecular ion	Naringenin hexoside I	Flavanone	(Xu et al. 2022)
		271 [580-H-162]$^-$	Loss of a hexose			
		151 [580-H-308-157]$^-$	Derived from the retro Diels-Alder mechanism			
18	29.99	609 [610-H]$^-$	Deprotonated molecular ion	Rutin	Flavonol	(Ramírez et al. 2020)
		301 [610-H-308]$^-$	Loss of a rutinoside group			
		151 [580-H-308-150]$^-$	Derived from the retro Diels-Alder mechanism			
19	30.68	609 [610-H]$^-$	Deprotonated molecular ion	Hesperidin	Flavanone	(Taamalli et al. 2015)
		301 [609-H-308]$^-$	Loss of a rutinoside group			
20	32.16	433 [434-H]$^-$	Deprotonated molecular ion	Naringenin hexoside II	Flavanone	(Xu et al. 2022)
		271 [580-H-162]$^-$	Loss of a hexose			
		151 [580-H-308-157]$^-$	Derived from the retro Diels-Alder mechanism			
21	32.89	385 [386-H]$^-$	Deprotonated molecular ion	Diferulic Acid	Hydroxycinnamic acid	(Félix-Medina et al. 2021)
		193 [356-H-192]$^-$	Loss of remains of a ferulic acid molecule			
		149 [356-H-192-44]$^-$	Loss of a CO_2 molecule			
22	33.7	389 [390-H]$^-$	Deprotonated molecular ion	5-hydroxy-penta-methoxy-flavone I	Flavone	(Xu et al. 2022)
		371 [390-H-18]$^-$	Loss of a H_2O molecule			
		353 [390-H-18-18]$^-$	Loss of a H_2O molecule			
		193 [390-H-18-18-32-32-32-32-32]$^-$	Loss of five methoxyl groups			

No.	RT (min)	Fragment ion	Fragment description	Identified compound	Compound type	Reference
23	34.4	577 [578-H]$^-$	Deprotonated molecular ion	Hydrotriferulic acid (Cyclic)	Hydroxycinnamic acid	(Ramírez et al. 2020)
		401 [578-H-192-44]$^-$	Loss of remains of a ferulic acid molecule and a CO_2 molecule			
		342 [578-H-192-44-44-15]$^-$	Loss of a CO_2 molecule and a methyl			
		144 [578-H-192-44-44-15-198]$^-$	Loss of remains of a hydroferulic acid molecule			
24	36.24	487 [488-H]$^-$	Deprotonated molecular ion	Caffeoyl-hexose-deoxyhexose	Hydroxycinnamic acid	(Chen et al. 2012)
		341 [488-H-146]$^-$	Loss of residues of a deoxy-hexose			
		179 [342-H-162]$^-$	Loss of a hexose			
25	37.35	371 [372-H]$^-$	Deprotonated molecular ion	Tangeretin	Flavone	(Wang et al. 2022)
		353 [372-H-18]$^-$	Loss of a H_2O molecule			
		225 [372-H-18-32-32-32-32]$^-$	Loss of four methoxyl groups			
		197 [372-H-18-32-32-32-32-28]$^-$	Loss of a carbonyl			
26	38.4	385 [386-H]$^-$	Deprotonated molecular ion	Hydrodiferulic acid	Hydroxycinnamic acid	(Ramírez et al. 2020)
		193 [386-H-192]$^-$	Loss of remains of a ferulic acid molecule			
		134 [386-H-192-44-15]$^-$	Loss of a CO_2 molecule and a methyl			

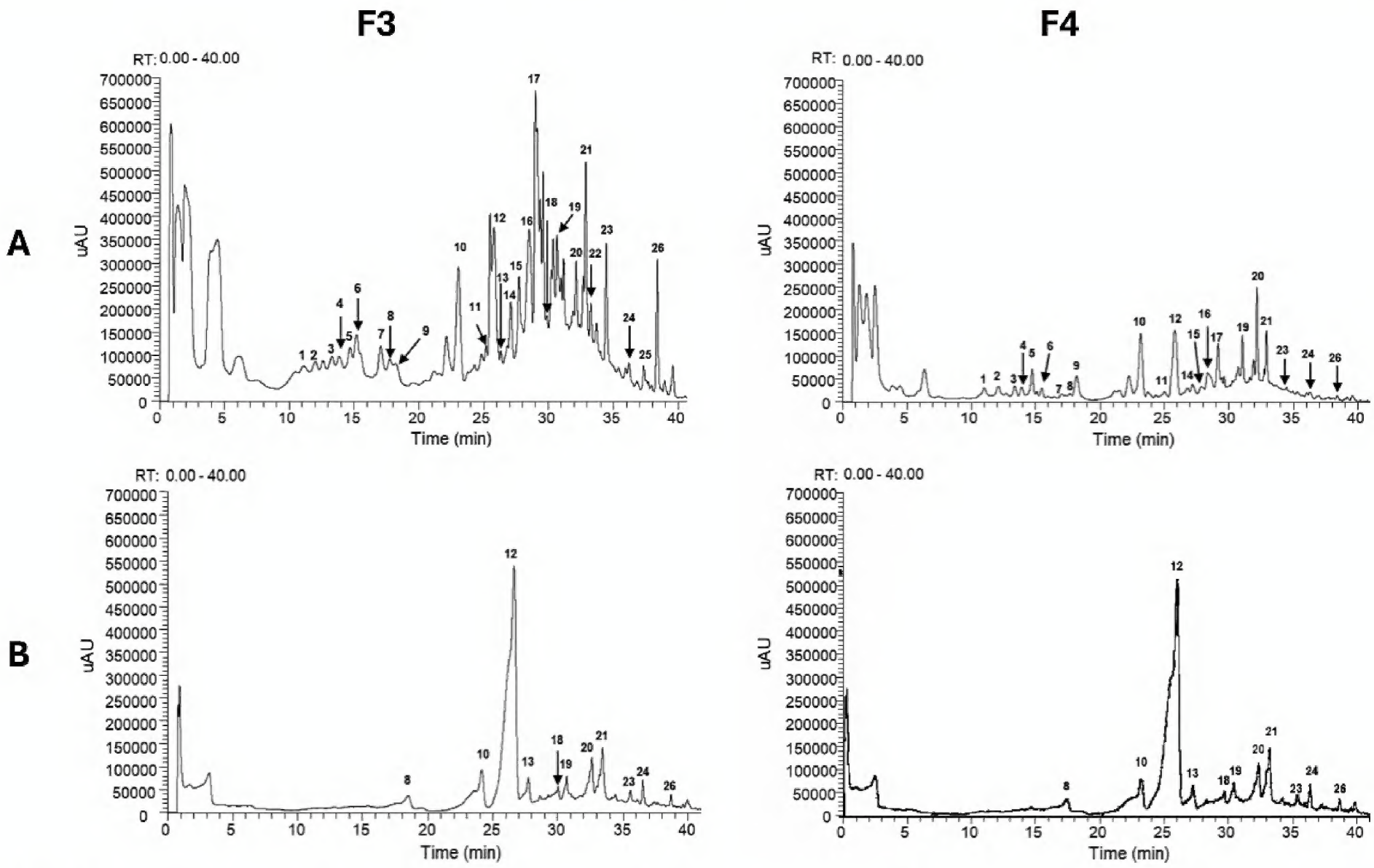

Figure 4. Representative UPLC chromatograms for the phenolic compounds content in the free (**A**) and bound (**B**) phytochemical fractions of indirectly expanded snack foods in PS3 obtained from formulation F3 (corn starch, whole yellow-corn flour, *Citrus mitis* by-products, skimmed milk powder) and formulation F4 (corn starch, whole yellow-corn flour, skimmed milk powder).

an overall acceptance of 54%, and the commercial product (COM) had an overall acceptance of 72%. However, when participants were presented with detailed information on the nutritional and antioxidant content of each formulation, F3 showed an 83% acceptance, F4 had 77%, and the commercial product (COM) reached an overall acceptance of 90%. For the flavor attribute, when information about the nutritional and antioxidant properties of the snack foods was not given to the panelists, acceptability values of 63%, 45%, and 71% were obtained for F3, F4, and COM, respectively. Nevertheless, the acceptability values in the snacks increased to 67% in F3, 61% in F4, and 83% in COM when nutritional information of the formulation was supplied to the participants. Likewise, for the color attribute, the snacks evaluated without providing prior nutritional information to evaluators presented acceptability values of 67%, 59%, and 66% for F3, F4, and COM, respectively. Nonetheless, the acceptability values for the color attribute were higher (82% in F3, 77% in F4, and 80% in COM) in snacks where the nutritional information was given to the panelists. Furthermore, for the texture attribute, the snack foods evaluated by panelists without providing prior nutritional information showed acceptability values of 80%, 66%, and 82% for F3, F4, and

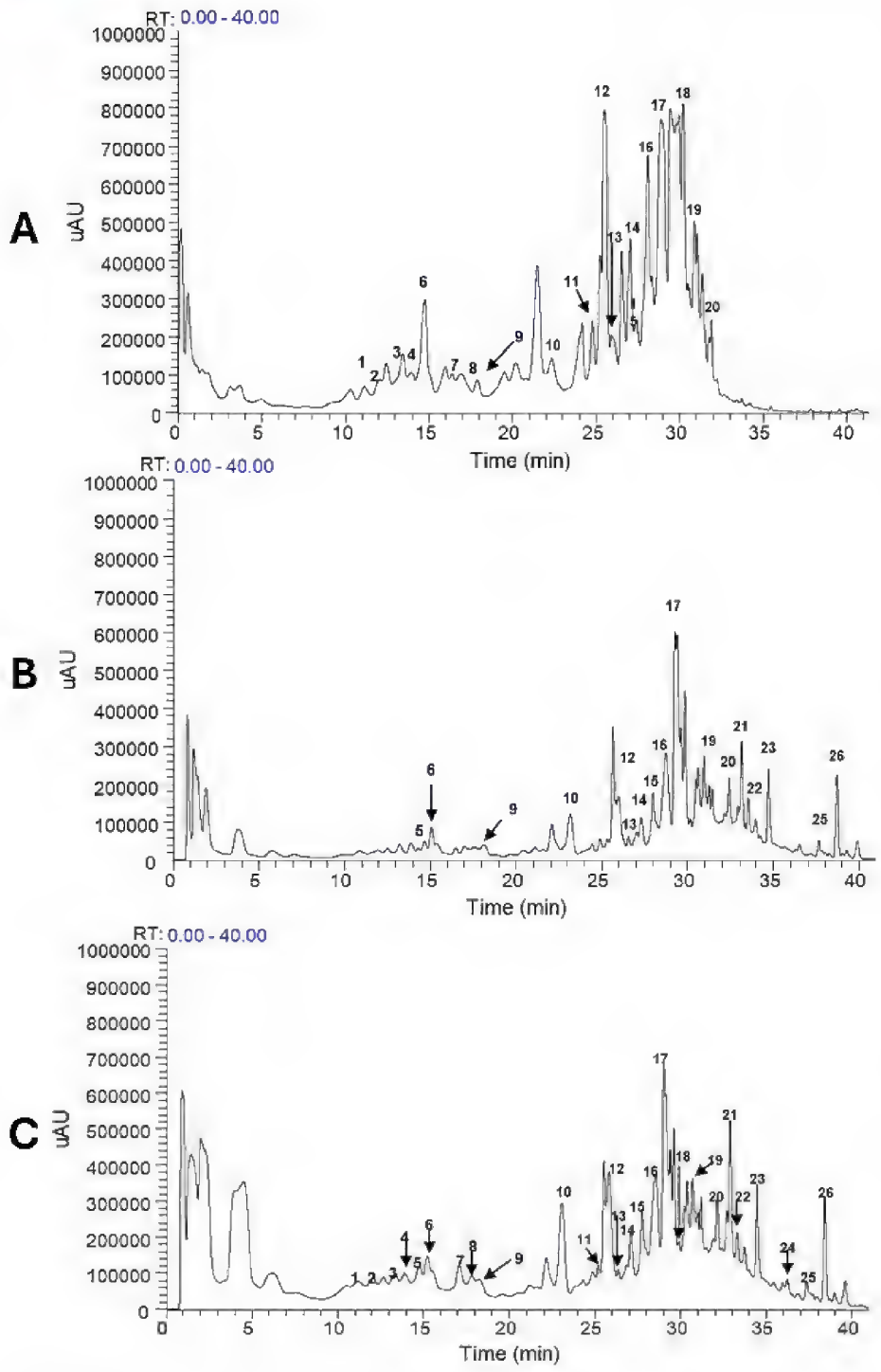

Figure 5. Profile of the phenolic compounds in the free phytochemical fraction in the different processing stages for the production of indirectly expanded snack foods (PS1 = unprocessed mixture, PS2 = extruded pellet, and PS3 = expanded snack) in formulation F3 (corn starch, whole yellow-corn flour, *Citrus mitis* by-products, skimmed milk powder).

COM, respectively. However, elevated acceptability values for the texture attribute were obtained (87% in F3, 87% in F4, and 89% in COM) when the nutritional information was supplied to the evaluators. These results highlight the significant influence ($p < 0.01$) of nutritional and antioxidant information on the overall acceptance of snacks. Participants demonstrated a greater preference for F3, F4, and COM when provided detailed information about their nutritional and antioxidant profiles, indicating a greater appreciation for products that offer better health benefits. Regarding flavor and texture attributes, the snacks from treatments F3 and F4 presented acceptable values by the panelists; however, these values were lower than those obtained by COM. This behavior could be because commercial products may present artificial flavorings, improving their flavor properties. However, extruded foods must conserve their natural taste and reduce the addition of flavorings to diminish public health problems such as obesity (Dos Santos et al. 2019). Also, the high acceptance values for the color attribute in F3 concerning F4 and COM were attributed to the attractive orange-yellow coloration presented by the *Citrus mitis* by-products. The overall acceptance of the snacks (PS3) obtained with the formulations F3 and F4, as well as a COM, was reported in a previous study (Ruiz-Armenta et al. 2019). In the treatment F3, around 65% of the panelists indicated a degree of acceptance from the hedonic scale ≥5. Also, approximately 75% of the panelists choose values ≥5 for the F4 snack. The degree of acceptance of the F4 formulation was close to that obtained in the commercial product, where nearly 80% of the panelists indicated acceptance values ≥5. These results were deemed positive due to the high amounts of antioxidant compounds and dietary fiber provided by *Citrus mitis* by-products. A study conducted by Delgado-Nieblas et al. (2017) reported that including *Citrus mitis* by-products in the production of extruded breakfast cereals from a mixture of wheat-bran, oat-bran, and yellow-corn-grits improved the sensory qualities of color and texture compared to a control product that did not contain *Citrus mitis*. Additionally, including these by-products did not significantly affect the overall acceptance of the product.

Essential amino acids (aa)

Amino acids are essential for human nutrition and health. These organic compounds are regarded as the fundamental building blocks of life because they are the structural units of proteins (López and Mohiuddin 2024). Amino acids have functions in broad physiological processes, such as cell signaling, genetic expression, DNA synthesis, and immune response. The adequate intake of amino acids is essential for health since it leads to the proper functioning of tissues and organic systems, and their insufficient consumption is associated with numerous disorders (Ryan et al. 2021). The protein content in extruded products depends on the quality and quantity of raw material used and exhibits considerable variation during processing (Sahoo et al. 2022). Histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and valine (Val) are identified as essential amino acids due to the inability of human and mammalian cells to synthesize them. Consequently, these amino acids must be obtained through dietary intake. The changes in the essential amino acids content were evaluated, considering only the unprocessed mixture (PS1) and the expanded snack (PS3) for F3 due to the quality characteristics (Ruiz-Armenta et al. 2019) and content of phytochemicals and antioxidants observed in this formulation, to know the nutritional contribution in the final product, ready for consumption. Table 2 shows the content of essential aa of the treatment F3 in the processing stages PS1 and PS3. According to FAO (2011), PS1 satisfies the daily requirement established for older children, adolescents, and adults. When the unprocessed mixture (PS1) was subjected to the extrusion process (PS2) and subsequently to microwave heating (PS3), pronounced changes in the aa profile were found. In stage PS3, the values

Table 2. Essential amino acids content and daily intake requirements (F3) in the processing stages (PS1 and PS3).

Amino acid	F3				FAO*
	PS1		PS3		
	mg/g protein	*Chemical Score*	*mg/g protein*	*Chemical score*	
Histidine (His)	44.82	280.13	39.7	248.13	16.0
Isoleucine (Ile)	32.61	108.70	20.11	67.03	30.0
Leucine (Leu)	132.47	217.16	92.8	152.13	61.0
Lysine (Lys)	79.09	164.77	36.48	76.00	48.0
Methionine (Met)	24.14	104.96	17.38	75.57	23.0
Threonine (Thr)	44.79	179.16	29.75	119.00	25.0
Valine (Val)	48.19	120.48	30.39	75.98	40.0
Phenylalanine (Phe)	53.61	130.76	36.82	89.80	41.0
Tryptophan (Trp)	14.10	213.64	10.45	158.33	6.6

*Daily requirements of amino acids (mg/g protein) for older children, adolescents, and adults according to FAO (2011); F3 = corn starch, whole yellow-corn flour, *Citrus mitis* by-products, skimmed milk powder; PS1 = unprocessed mixture; PS3 = expanded snack.

obtained for Ile, Lys, Met, Val, and Phe were lower than the daily requirements of amino acids for older children, adolescents, and adults, according to FAO (2011). Lys presented the lowest stability during processing (46% retention). Similar results were reported by Ilo and Berghofer (2003) (Lys' retentions of 51–89%) in extruded corn grits. The lysine ε-amino group has been considered the principal reactant in the Maillard reaction. Also, the starch and protein degradation during the extrusion process favors browning reactions due to the exposure of reactive sites (Mauron 1990). Therefore, a higher concentration of skimmed milk powder in F3 is suggested to obtain snack foods with higher protein quality and a better nutritional contribution. Román-Gutiérrez et al. (2021) produced barley/corn snacks and observed that the concentrations of serine, glycine, histidine, threonine, methionine, cysteine, isoleucine, and leucine increased significantly ($p < 0.05$) after the extrusion process. According to these authors, the protein structure is modified during the extrusion process, and the intermolecular disulfide bonds and the hydrogen bonds are broken, generating a higher protein degradation that allows more amino acids to be available. Sahoo et al. (2022) found that the addition of bean powder increased the content of lysine, leucine, isoleucine, cysteine, threonine, tyrosine, and methionine in corn-based snacks. Likewise, they observed a decrease in glutamate, serine, glycine, arginine, alanine, valine, and phenylalanine.

Conclusions

The different processing stages studied in the production of indirectly expanded snack foods significantly affected the different characteristics of the obtained products. Likewise, the addition of raw materials with high phytochemicals content, such as whole yellow corn flour and *Citrus mitis* by-products, as well as the incorporation of skimmed milk powder in the production of indirectly expanded snack foods, was an adequate alternative to improve its bioactive compounds content and quality protein content, respectively. The snack foods presented acceptable sensory properties. In addition, processing techniques such as extrusion and microwave heating can release phenolic compounds, providing higher antioxidant activity and producing healthy snack foods with high functional potential, presenting better nutritional and functional characteristics than commercial products. The future perspectives of the present study are to perform glycemic index measurements in persons who consume snack foods due to the important contribution of dietary fiber provided by *Citrus mitis* by-products added to the F3 formulation. These measurements would demonstrate health benefits in people who present diabetes disease since it is a public health problem in many countries worldwide. Another perspective is to conduct *in vivo* tests on the anticholesterolemic effect of consuming snack foods by adding *Citrus mitis* by-products that present possible benefits in reducing the incidence of cardiovascular diseases.

Author contributions

X.A.R-A and C.I.D-N.: Investigation, Methodology, Data Analysis, Writing-original draft. J.J.Z-M.: Design of experiments, Supervision, Visualization, and Project Administration. E.A-P., P.R.F-V.: Technical assistance and Data Analysis. J.A.G-I., J.A.L-V, M.F.Q-S.: Technical assistance in phytochemical and antioxidant analyses. All authors read and approved the final manuscript.

Funding

This work was supported by PROFAPI-2015-087-UAS.

Conflict of interest

The authors have no conflicts of interest to declare.

Financial interests

All authors endorse that they have no affiliations or collaboration with any organization with any financial interest in the materials discussed in this manuscript.

Acknowledgements

The authors thank CONAHCYT and PROFAPI-2015-087-UAS for the financial support granted for the doctoral studies of Ph.D. Xóchitl Ariadna Ruiz Armenta, INSTITUTO TECNOLÓGICO DE DURANGO, for lending its laboratories to carry out the ILDLox and HRSA analyses, and INCMN SALVADOR ZUBIRÁN for lending its laboratories to perform the essential amino acids determination.

References

Adom KK, Liu RH (2002) Antioxidant activity of grains. Journal of Agricultural and Food Chemistry 50(21): 6182–6187. https://doi.org/10.1021/jf0205099

Alam MN, Bristi NJ, Rafiquzzaman M (2013) Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharmaceutical Journal 21(2): 143–152. https://doi.org/10.1016/j.jsps.2012.05.002

Barraza-Elenes C, Camacho-Hernández IL, Yahia EM, Zazueta-Morales JJ, Aguilar-Palazuelos E, Heredia JB, Muy-Rangel D, Delgado-Nieblas CI, Carrillo-López A (2019) Analysis by UPLC-DAD-ESI-MS of phenolic compounds and HPLC-DAD based determination of carotenoids in noni (*Morinda citrifolia* L.) bagasse. Journal of Agricultural and Food Chemistry 67: 7365–7377. https://doi.org/10.1021/acs.jafc.9b02716

Brennan C, Brennan M, Derbyshire E, Tiwari BK (2011) Effects of extrusion on the polyphenols, vitamins and antioxidant activity of foods. Trends in Food Science & Technology 22(10): 570–575. https://doi.org/10.1016/j.tifs.2011.05.007

Buitimea-Cantúa NE, Torres-Chávez PI, Ramírez-Wong B, Ledesma-Osuna AI, Gutiérrez-Uribe JA, Serna-Guerrero DA, Serna-Saldívar SO (2018) Ferulic, *p*-coumaric, diferulic and triferulic acids contents of corn tortillas prepared with extruded corn flour and enriched with sorghum (*Sorghum bicolor* (L.) Moench) bran. Journal of Food Measurement and Characterization 12: 1633–1640. https://doi.org/10.1007/s11694-018-9778-4

Castro-Montoya YA, Jacobo-Valenzuela N, Delgado-Nieblas CI, Ruiz-Armenta XA, Heredia JB, Delgado-Murillo SA, Calderón-Castro A, Zazueta-Morales JJ (2024) Effect of the extrusion process on phytochemical, antioxidant, and cooking properties of gluten-free pasta made from broken rice and nopal. Revista Mexicana de Ingeniería Química 23: Alim24149. https://doi.org/10.24275/rmiq/Alim24149

Chen HJ, Inbaraj BS, Chen BH (2012) Determination of phenolic acids and flavonoids in *Taraxacum formosanum* Kitam by liquid chromatography-tandem mass spectrometry coupled with a post-column derivatization technique. International Journal of Molecular Sciences 13: 260–285. https://doi.org/10.3390/ijms13010260

Cheng JC, Dai F, Zhou B, Yang L, Liu ZL (2007) Antioxidant activity of hydroxycinnamic acid derivatives in human low density lipoprotein: mechanism and structure–activity relationship. Food Chemistry 104: 132–129. https://doi.org/10.1016/j.foodchem.2006.11.012

Delgado-Nieblas CI, Zazueta-Morales JJ, Ahumada-Aguilar JA, Aguilar-Palazuelos E, Carrillo-López A, Jacobo-Valenzuela N, Telis-Romero J (2017) Optimization of an air-drying process to obtain a dehydrated naranjita (*Citrus mitis* B.) pomace product with high bioactive compounds and antioxidant capacity. Journal of Food Process Engineering 40(1): e12338. https://doi.org/10.1111/jfpe.12338

Dewanto V, Wu X, Adom KK, Liu RH (2002) Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry 50(10): 3010–3014. https://doi.org/10.1021/jf0115589

Dos Santos PA, Caliari M, Júnior MSS, Silva KS, Viana LF, Garcia LGC, de Lima MS (2019) Use of agricultural by-products in extruded gluten-free breakfast cereals. Food Chemistry 124956. https://doi.org/10.1016/j.foodchem.2019.124956

FAO (2011) Dietary protein quality evaluation in human nutrition. FAO Food and Nutrition Paper. Auckland, New Zealand. [ISSN 0254-4725]

Félix-Medina JV, Gutiérrez-Dorado R, López-Valenzuela JA, López-Angulo G, Quintero-Soto MF, Perales-Sánchez JXK, Montes-Ávila J (2021) Nutritional, antioxidant and phytochemical characterization of healthy ready-to-eat expanded snack produced from maize/common bean mixture by extrusion. LWT 142: 111053. https://doi.org/10.1016/j.lwt.2021.111053

Gamboa-Gómez CI, Muñoz-Martínez A, Rocha-Guzmán NE, Gallegos-Infante JA, Moreno-Jiménez MR, González-Herrera SM, Soto-Cruz O, González-Laredo RF (2016) Changes in phytochemical and antioxidant potential of tempeh common bean flour from two selected cultivars influenced by temperature and fermentation time. Journal of Food Processing and Preservation 40(2): 270–278. https://doi.org/10.1111/jfpp.12604

Gąsecka A, Rogula S, Szarpak Ł, Filipiak KJ (2021) LDL-cholesterol and platelets: insights into their interactions in atherosclerosis. Life 11(1): 39. https://doi.org/10.3390/life11010039

Gaxiola-Cuevas N, Mora-Rochín S, Cuevas-Rodríguez EO, León-López L, Reyes-Moreno C, Montoya-Rodríguez A, Milán-Carrillo J (2017) Phenolic acids profiles and cellular antioxidant activity in tortillas produced from mexican maize landrace processed by nixtamalization and lime extrusion cooking. Plant Foods for Human Nutrition 72(3): 314–320. https://doi.org/10.1007/s11130-017-0624-3

Gümüşay ÖA, Şeker M (2021) Effects of extrusion parameters on physicochemical properties of third generation corn snacks expanded by microwave heating. Journal of Food Processing and Preservation 45(7): e15630. https://doi.org/10.1111/jfpp.15630

Hsu B, Coupar IM, Ng K (2006) Antioxidant activity of hot water extract from the fruit of the *Doum palm*, *Hyphaene thebaica*. Food Chemistry 98(2): 317–328. https://doi.org/10.1016/j.foodchem.2005.05.077

Ilo S, Berghofer E (2003) Kinetics of lysine and other amino acids loss during extrusion cooking of maize grits. Journal of Food Science 68(2): 496–502. https://doi.org/10.1111/j.1365-2621.2003.tb05701.x

Kiokias S, Proestos C, Oreopoulou V (2018) Effect of natural food antioxidants against LDL and DNA oxidative changes. Antioxidants 7(10): 133. https://doi.org/10.3390/antiox7100133

Li D, Rui YX, Guo SD, Luan F, Liu R, Zeng N (2021) Ferulic acid: A review of its pharmacology, pharmacokinetics and derivatives. Life Sciences 284: 119921. https://doi.org/10.1016/j.lfs.2021.119921

Lim J (2011) Hedonic scaling: A review of methods and theory. Food Quality and Preference 22: 733–747. https://doi.org/10.1016/j.foodqual.2011.05.008

Lopez MJ, Mohiuddin SS (2024) Biochemistry, Essential Amino Acids. In: StatPearls. https://www.ncbi.nlm.nih.gov/books/NBK557845/?report=classic

Lou SN, Hsu YS, Ho CT (2014) Flavonoid compositions and antioxidant activity of calamondin extracts prepared using different solvents. Journal of Food and Drug Analysis 22(3): 290–295. https://doi.org/10.1016/j.jfda.2014.01.020

Maetens E, Hettiarachchy N, Dewettinck K, Horax R, Moens K, Moseley DO (2017) Physicochemical and nutritional properties of a healthy snack chip developed from germinated soybeans. LWT 84: 505–510. https://doi.org/10.1016/j.lwt.2017.06.020

Martemucci G, Costagliola C, Mariano M, D'andrea L, Napolitano P, D'Alessandro AG (2022) Free radical properties, source and targets, antioxidant consumption and health. Oxygen 2(2): 48–78. https://doi.org/10.3390/oxygen2020006

Mauron J (1990) Influence of processing on protein quality. Journal of Nutritional Science and Vitaminology 36(4-SupplementI): S57–S69. https://doi.org/10.3177/jnsv.36.4-SupplementI_S57

Moreno-Jiménez MR, Cervantes-Cardoza V, Gallegos-Infante JA, González-Laredo RF, Estrella I, García-Gasca TJ, Herrera-Carrera E, Díaz-Rivas, JO, Rocha-Guzmán NE (2015) Phenolic composition changes of processed common beans: Their antioxidant and anti-inflammatory effects in intestinal cancer cells. Food Research International 76: 79–85. https://doi.org/10.1016/j.foodres.2014.12.003

Morales-De León J, Bourges H, Camacho ME (2005) Amino acid composition of some Mexican foods. Archivos Latinoamericanos de Nutrición 55(1): 172–186.

Moulehi I, Bourgou S, Ourghemmi I, Tounsi MS (2012) Variety and ripening impact on phenolic composition and antioxidant activity of mandarin (*Citrus reticulate* Blanco) and bitter orange (*Citrus aurantium* L.) seeds extracts. Industrial Crops and Products 39: 74–80. https://doi.org/10.1016/j.indcrop.2012.02.013

Panak-Balentić J, Jozinović A, Ačkar Đ, Babić J, Miličević B, Benšić M, Jokić S, Šarić A, Šubarić D (2019) Nutritionally improved third generation snacks produced by supercritical CO_2 extrusion I. Physical and sensory properties. Journal of Food Process Engineering 42(2): e12961. https://doi.org/10.1111/jfpe.12961

Patil SS, Varghese E, Rudra SG, Kaur C (2016) Effect of extrusion processing on phenolics, flavonoids and antioxidant activity of millets. International Journal of Food and Fermentation Technology 6(1): 177–184. https://doi.org/10.5958/2277-9396.2016.00040.4

Pineda-Hidalgo KV, Flores-Leyva B, Salazar-Salas NY, Chávez-Ontiveros J, Garzón-Tiznado JA, Sánchez-López J, Delgado-Vargas F, López-Valenzuela JA (2022) Expression of MYB transcription factors and target genes and its association with phenolic content and antioxidant activity of selected *Solanum lycopersicum* var. *cerasiforme* accessions from Mexico. CyTA-Journal of Food 20: 374–384. https://doi.org/10.1080/19476337.2022.2144951

Poljsak B, Kovač V, Milisav I (2021) Antioxidants, food processing and health. Antioxidants 10(3): 433. https://doi.org/10.3390/antiox10030433

Qiao H, Shao H, Zheng X, Liu J, Liu J, Huang J, Zhang C, Liu Z, Wang J, Guan W (2021) Modification of sweet potato (*Ipomoea batatas* Lam.) residues soluble dietary fiber following twin-screw extrusion. Food Chemistry 335: 127522. https://doi.org/10.1016/j.foodchem.2020.127522

Quintero-Soto MF, Saracho-Peña AG, Chávez-Ontiveros J, Garzón-Tiznado JA, Pineda-Hidalgo KV, Delgado-Vargas F, López-Valenzuela JA (2018) Phenolic profiles and their contribution to the antioxidant activity of selected chickpea genotypes from Mexico and ICRISAT collections. Plant Foods for Human Nutrition 73: 122–129. https://doi.org/10.1007/s11130-018-0661-6

Quintero-Soto MF, Espinoza-Moreno RJ, Félix-Medina JV, Salas-López F, López-Carrera CF, Argüelles-López OD, Vázquez-Ontiveros ME, Gómez-Favela MA (2022) Comparison of phytochemical profile and *in vitro* bioactivity of beverages based on the unprocessed and extruded sesame (*Sesamum indicum* L.) seed byproduct. Foods 11: 3175. https://doi.org/10.3390/foods11203175

Rahman MA, Abdullah N, Aminudin N (2015) Antioxidative effects and inhibition of human low density lipoprotein oxidation *in vitro* of polyphenolic compounds in *Flammulina velutipes* (Golden Needle Mushroom). Oxidative Medicine and Cellular Longevity 2015: 403023. https://doi.org/10.1155/2015/403023

Ramírez K, Quintero-Soto MF, Rochín-Medina JJ (2020) Enhancement of the antioxidant and antimicrobial activities of maize wastewater by an eco-friendly process. Journal of Food Measurement and Characterization 14: 1682–1689. https://doi.org/10.1007/s11694-020-00416-1

Rao PV, Kiran SDVS, Rohini P, Bhagyasree P (2017) Flavonoid: A review on Naringenin. Journal of Pharmacognosy and Phytochemistry 6(5): 2778–2783.

Rocha-Guzmán NE, Gallegos-Infante JA, Delgado-Nieblas CI, Zazueta-Morales JJ, González-Laredo RF, Cervantes-Cardoza V, Martínez-Bustos F, Aguilar-Palazuelos E (2012) Effect of extrusion cooking on the antioxidant activity of extruded half product snacks made of yellow-corn and pumpkin flours. International Journal of Food Engineering 8(4): 1–13. https://doi.org/10.1515/1556-3758.2284

Román-Gutiérrez AD, Barrera-Barrera DI, Morales-Sánchez E, Castro-Rosas J, Gómez-Aldapa CA, Falfán-Cortés RN, Rodríguez-Martínez ML, Guzmán-Ortiz FA (2022) Modelling and optimization of the extrusion process in a snack of barley/corn and nutritional evaluation of the optimized product. Cereal Chemistry 99(3): 556–567. https://doi.org/10.1002/cche.10518

Routray W, Orsat V (2012) Microwave-assisted extraction of flavonoids: a review. Food and Bioprocess Technology 5: 409–424. https://doi.org/10.1007/s11947-011-0573-z

Ruiz-Armenta XA, Zazueta-Morales JJ, Delgado-Nieblas CI, Carrillo-López A, Aguilar-Palazuelos E, Camacho-Hernández IL (2019) Effect of the extrusion process and expansion by microwave heating on physicochemical, phytochemical, and antioxidant properties during the production of indirectly expanded snack foods. Journal of Food Processing and Preservation 43: e14261. https://doi.org/10.1111/jfpp.14261

Ryan PJ, Riechman SE, Fluckey JD, Wu G (2021) Interorgan metabolism of amino acids in human health and disease. In: Wu G (Ed.) Amino Acids in Nutrition and Health. Advances in Experimental Medicine and Biology, vol 1332. Springer, Cham. https://doi.org/10.1007/978-3-030-74180-8_8

Safe S, Jayaraman A, Chapkin RS, Howard M, Mohankumar K, Shrestha R (2021) Flavonoids: structure-function and mechanisms of action and opportunities for drug development. Toxicological Research 37(2): 147–162. https://doi.org/10.1007/s43188-020-00080-z

Sahoo MR, Kuna A, Devi MP, Sowmya M, Dasgupta M (2022) Fortification of ready-to-eat extruded snacks with tree bean powder: nutritional, antioxidant, essential amino acids, and sensory properties. Journal of Food Science and Technology 59: 2351–2360. https://doi.org/10.1007/s13197-021-05251-w

Simirgiotis M, Benites J, Areche C, Sepúlveda B (2015) Antioxidant capacities and analysis of phenolic compounds in three endemic *Nolana* species by HPLC-PDA-ESI-MS. Molecules 20(6): 11490–1157. https://doi.org/10.3390/molecules200611490

Taamalli A, Arráez-Román D, Abaza L, Iswaldi I, Fernández-Gutiérrez A, Zarrouk M, Segura-Carretero A (2015) LC-MS-based metabolite profiling of methanolic extracts from the medicinal and aromatic species *Mentha pulegium* and *Origanum majorana*. Phytochemical Analysis 26(5): 320–330. https://doi.org/10.1002/pca.2566

Wang Z, Yan L, Ning T, Wang X, Li R, Zhang H (2020) Increasing soluble dietary fiber content and antioxidant activity of wheat bran through twin-screw extrusion pretreatment. Preparative Biochemistry & Biotechnology 50(9): 954–960. https://doi.org/10.1080/10826068.2020.1777424

Wang Z, Yang B, Chen X, Huang P, Chen K, Ma Y, Agarry IE, Kan J (2022) Optimization and comparison of nonconventional extraction techniques for soluble phenolic compounds from brocade orange (*Citrus sinensis*) peels. Journal of Food Science 87: 4917–4929. https://doi.org/10.1111/1750-3841.16356

Wani SA, Kumar P (2016) Effect of extrusion on the nutritional, antioxidant and microstructural characteristics of nutritionally enriched snacks. Journal of Food Processing and Preservation 40(2): 166–173. https://doi.org/10.1111/jfpp.12593

Wani SA, Ganie NA, Kumar P (2021) Quality characteristics, fatty acid profile and glycemic index of extrusion processed snacks enriched with the multicomponent mixture of cereals and legumes. Legume Science 3(2): e76. https://doi.org/10.1002/leg3.76

WHO (2020) World Health Organization. World Health Statistics 2020: Monitoring health for the SDGs, Sustainable Development Goal. [ISBN: 9789240011953]

Xu E, Wu Z, Pan X, Long J, Wang F, Xu X, Jin Z, Jiao A (2016) Effect of enzymatic (thermostable α-amylase) treatment on the physicochemical and antioxidant properties of extruded rice incorporated with soybean flour. Food Chemistry 197: 114–123. https://doi.org/10.1016/j.foodchem.2015.10.109

Xu M, Yi L, Ren D, Li B (2022) Mass defect filtering combined with molecular networking to profile flavonoids in citrus fruit based on liquid chromatography-high resolution mass spectrometry platform: *Citrus sinensis* (L.) Osbeck as a case study. Journal of Chromatography A 1685: 463640. https://doi.org/10.1016/j.chroma.2022.463640

Zeng Z, Liu C, Luo S, Chen J, Gong E (2016) The profile and bioaccessibility of phenolic compounds in cereals influenced by improved extrusion cooking treatment. PLOS ONE 11(8): e0161086. https://doi.org/10.1371/journal.pone.0161086

Zielinski H, Kozlowska H, Lewczuk B (2001) Bioactive compounds in the cereal grains before and after hydrothermal processing. Innovative Food Science & Emerging Technologies 2(3): 159–169. https://doi.org/10.1016/S1466-8564(01)00040-6

Žilić S, Serpen A, Akıllıoğlu G, Gökmen V, Vančetović J (2012) Phenolic compounds, carotenoids, anthocyanins, and antioxidant capacity of colored maize (*Zea mays* L.) kernels. Journal of Agricultural and Food Chemistry 60(5): 1224–1231. https://doi.org/10.1021/jf204367z